Improving Gene Editing Outcomes in Human Hematopoietic Stem and Progenitor Cells by Temporal Control of DNA Repair.

Journal: Stem Cells

Publication Year: 2018

Authors: Anastasia Lomova, Danielle N Clark, Beatriz Campo-Fernandez, Carmen Flores-Bjurstrom, Michael L Kaufman, Sorel Fitz-Gibbon, Xiaoyan Wang, Eric Y Miyahira, Devin Brown, Mark A DeWitt, Jacob E Corn, Roger P Hollis, Zulema Romero, Donald B Kohn

PubMed link: 30372555

Funding Grants: Beta-Globin Gene Correction of Sickle Cell Disease in Hematopoietic Stem Cells, Curing Sickle cell Disease with CRISPR-Cas9 genome editing

Public Summary:
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated system (Cas9)-mediated gene editing of human hematopoietic stem cells (hHSCs) is a promising strategy for the treatment of genetic blood diseases through site-specific correction of identified causal mutations. However, clinical translation is hindered by low ratio of precise gene modification using the corrective donor template (homology-directed repair, HDR) to gene disruption (nonho- mologous end joining, NHEJ) in hHSCs. By using a modified version of Cas9 with reduced nucle- ase activity in G1 phase of cell cycle when HDR cannot occur, and transiently increasing the proportion of cells in HDR-preferred phases (S/G2), we achieved a four-fold improvement in HDR/NHEJ ratio over the control condition in vitro, and a significant improvement after xeno- transplantation of edited hHSCs into immunodeficient mice. This strategy for improving gene editing outcomes in hHSCs has important implications for the field of gene therapy, and can be applied to diseases where increased HDR/NHEJ ratio is critical for therapeutic success.

Scientific Abstract:
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated system (Cas9)-mediated gene editing of human hematopoietic stem cells (hHSCs) is a promising strategy for the treatment of genetic blood diseases through site-specific correction of identified causal mutations. However, clinical translation is hindered by low ratio of precise gene modification using the corrective donor template (homology-directed repair, HDR) to gene disruption (nonhomologous end joining, NHEJ) in hHSCs. By using a modified version of Cas9 with reduced nuclease activity in G1 phase of cell cycle when HDR cannot occur, and transiently increasing the proportion of cells in HDR-preferred phases (S/G2), we achieved a four-fold improvement in HDR/NHEJ ratio over the control condition in vitro, and a significant improvement after xenotransplantation of edited hHSCs into immunodeficient mice. This strategy for improving gene editing outcomes in hHSCs has important implications for the field of gene therapy, and can be applied to diseases where increased HDR/NHEJ ratio is critical for therapeutic success. Stem Cells 2018.